



FLORIDA CITRUS MUTUAL



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The Florida Citrus Research and Development Foundation, Inc. has released its 2023 year-end report and it is available below.



New Year Priorities and a Changing of the Guard

As this year grows to a close, it is a good time to recognize three outstanding CRDF board members who will be leaving the board because of term limits: David Howard, Rob Atchley, and John Updike. Howard and Atchley are former presidents, and each brought something special. Howard had a strong focus on plant breeding and ushered in the advent of “directed research,” a method of funding research that is quicker and more targeted. Atchley presided with a business-like approach, making sure the research we funded was not just an academic exercise. He also saw the entire field when deliberating. Updike chaired the Research Management Committee — the heart and soul of CRDF’s work. He read everything and was an encyclopedia of knowledge. He was always one of the smartest people in the room. I’m going to miss these gentlemen.

Taking their places will be Matt Machata, Sarah Spinosa and Trevor Murphy. Machata is a grower from the Lake Wales area and has an engineering background. Spinosa is involved with her family’s citrus operations and was once with the FDOC. Murphy is a grower with Kahn Citrus Management out of Sebring. Each has a skill set that will fit nicely with research projects we will be considering.

At its last board meeting, the CRDF board also decided the officers and committee

chairs for the new year. Morgan Porter of McKenna Brothers, Inc. will stay as president; Joby Sherrod of Alico, Inc. will be vice-president and chair the Plant Improvement Committee, a committee that will hopefully serve as an interface with UF/IFAS and USDA/ARS plant breeders we fund, helping to move germplasm through the plant breeding pipeline in a way that will give you confidence to buy new releases. George Hamner, a member of the Florida Citrus Hall of Fame, will serve as Secretary and also chair the Governance Committee. He knows the industry inside and out, so he will be well suited to ensure that CRDF is organized and positioned to best help the industry. Ron Mahan of Tamiami Citrus, LLC, will remain as treasurer and chair of the Finance & Audit Committee. Ron does a great job helping CRDF plan its funding needs to stay in line with its research portfolio and understands the citrus industry in macro terms as well as day-to-day operations of the business. Finally, but perhaps most important, Aaron Himrod will take over as chair of the Research Management Committee. This committee analyzes the hard research that address the needs of the Florida citrus industry, and it is hard research that will solve the existential threat of HLB. Aaron has earned his stripes as vice-chair for the last two years and is ready.

I hope you will agree that CRDF is ready for the challenges of the new year. So, what does the new year look like? We have three high priorities. The first is to continue researching ways to squeeze the most we can out of oxytetracycline (OTC) usage, either by funding research on ways to get more product into the phloem, combining it with other compounds that maximize OTC's efficacy, or help the tree fight CLAs or other pathogens. The second is finding molecules or compounds that could take the place of OTC if the bacterium becomes resistant to OTC or the trees become phytotoxic to the OTC solution or multiple injections. The third is funding the development of the "tree of the future," a tree that is sufficiently tolerant to greening or perhaps even resistant. CRDF is funding many projects on these priorities and is considering numerous others. These three priorities will not be all we consider – for example, we have two Diaprepes projects under consideration as I write this. We know we have to do something about Diaprepes because it is a "hair on fire" research need of Florida growers, and there will be others, but the three priorities highlighted above will be our focus as we start the new year.

CRDF has 63 projects under contract. Here are 12 projects worth keeping your eye on.

1. Drs. Randy Niedz and Michelle Heck, USDA/ARS, in Ft. Pierce and Ithaca, NY, and their teams are taking molecules or compounds identified by Dr. Brian Scully, retired USDA/ARS scientist, as possibly having antibacterial properties and injecting

them into trees of fruit bearing age to see how they compare with trees injected with oxytetracycline (OTC). The traditional scientific model would dictate that the molecules first be tested in an assay of sorts, then if it demonstrates sufficient antimicrobial activity against CLAs, test the molecule in a greenhouse before going to the field. However, this takes time, which is not on the side of growers, so Drs. Niedz and Heck bypassed all of this and went straight to the field. Dr. Niedz says, “how it performs in the field is the only assay that really matters.”

- So far, they have injected 88 molecules, 13 of which appear to be equal to or outperforming the OTC control trees, and Dr. Scully has found another 50 or so that need testing. This is important for several reasons, not the least of which is the need to have a backup ready if CLAs develops resistance to OTC. CRDF is contracting with grower-cooperators to test these molecules, covering certain costs and expenses.

2. Dr. Heck and her team are also working on a way to deliver antimicrobial peptides to citrus trees. Antimicrobial peptides are small proteins that can have the same effect as OTC, but which are typically much more expensive to produce. They call the approach Symbiont technology, which operates by modifying *Agrobacterium's* T-DNA, causing it to produce specific plant growth regulators and antimicrobial peptides. This results in a cluster of plant cells, referred to as Symbionts, that grow on a fixed site of the trunk of the citrus tree, to connect to the tree's vascular system, and continuously generate these antimicrobial peptides, which then flow into and distribute throughout the tree. The team has developed Symbionts for over 40 antimicrobial peptides and found four to be effective against HLB in initial greenhouse tests. Dr. Heck says, “Symbionts are to citrus trees what insulin pumps are for diabetic patients.” If successful, this approach should be affordable to growers as the Symbiont must only be grafted onto the tree once and the antimicrobial peptides are produced continuously throughout the Symbiont's life. They secured ten acres in the Indian River Citrus District, meeting EPA and APHIS criteria for field testing Symbionts.

3. The Bayer Crop Science project funded by CRDF, PepsiCo and Coca-Cola, and later with funding from the California Citrus Research Board and the USDA, has come to an end except for No Cost Extensions which allow researchers to wind up their work and spend their remaining funds. He's a summary of the project. The project had three objectives: to develop a high throughput screening cascade to evaluate antibacterial microbes and synthetic compounds for efficacy against HLB, to find a curative antibacterial microbe, and to develop a plant host defense solution using a synthetic chemical.

- At the end of the three-year project, the high throughput screening cascade

had been established, 2500 antibacterial microbes had been screened against pathogens, and 150,000 synthetic chemicals had been tested for inducing plant natural defenses (Plant Defense Inducers, or “PDIs”). Of those showing sufficient levels of efficacy, 6560 were tested in citrus. From these, top candidates were placed in four Florida field trials.

- During this three-year period, promising antibacterial microbial candidates were narrowed and synthetic PDIs were optimized leading to synthesis of 6000 PDI analogs. Top performers of both approaches were placed in field trials. Other scientists were also given access to the high throughput screening cascade that had been developed.

The project was then funded by a three-year USDA-NIFA grant. Despite testing 23 antibacterial microbial strains in greenhouse HLB positive trees, none were determined to be efficacious enough against liberibacter *and* have suitable toxicology profiles to believe they could receive registration approval from federal regulatory agencies. However, two synthetic PDIs show enough promise in greenhouse conditions to warrant further work. In one ongoing field trial, one of these analogs – especially when sprayed as a follow-up to an OTC injection – showed a good level of protection against HLB and tree performance was better than in those receiving only OTC treatment. This lead also gave protection against canker in leaf tests. If a decision is made to pursue further work on this PDI, the next step would likely be another year of field trials.

While not the homerun we had hoped to hit, I think it is fair to say the project was a solid double off the wall in left-center, especially if it leads to a new product that can be used on HLB *and* canker.

4. The board agreed to fund the testing of five molecules identified by Kranthi Mandadi of Texas A&M University as having high efficacy in killing CLAs. Mandadi developed the hairy root assay in which citrus roots are infected with HLB and potential killing agents are taken up by these roots. This allows the effectiveness of the killing agents to be measured. He has screened thousands of candidates and CRDF is funding the testing of five of the top ones. We believe these molecules can survive the federal regulatory process. They will be tested in the field by Ute Albrecht of the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS).

5. Second, Ozgur Batuman, UF/IFAS, was funded to inject five antimicrobials — including streptomycin — in rotation with OTC. Why streptomycin? It was discovered in earlier studies that there may be a synergistic effect between OTC and streptomycin, meaning that the reduction in titer was nearly 100%. High kill rates of bacteria are a good way to prevent resistance from developing.

6. Dr. Swadesh Santra, UCF, has developed an OTC injection mixture which is pH neutral. If this works, it could be a game-changer because a pH neutral solution may better allow pesticides and other products to be added to it and cause less damage to the tree. Residue testing will be key since a lower pH helps keep residue levels below regulatory thresholds, and the last thing we need to do is pursue products that don't work as well in this regard, but this product merits investigating.

7. The board also funded a proposal from Yu Wang, UF/IFAS, to test the injection of nearly 100 natural molecules that she has discovered have antibacterial effects. We don't know if they will work against CLAs, but if they do, it may provide an alternative to bactericides, which could have certain advantages in the marketplace.

8. CRDF has funded work with a peptide brought to us by the company Elemental Enzymes. The product has finally received its registration approval. It can be injected or sprayed, and works by switching on the plant's natural defenses. To my knowledge, never has CRDF had a company bring to us so much field data supporting its claim of efficacy against HLB. What is particularly exciting about this product is it can be used alongside OTC, so CRDF has agreed to pay for testing to see if the OTC knocks the level of CLAs bacteria down and the Elemental Enzymes product keeps reinfection rates down.

9. CRDF is funding work to determine if the use of Gibberellic acid and/or 2-4/D in concert with OTC will reduce fruit drop by more than OTC used by itself.

10. We are funding work with Drs. Zhonglin Mou and Manjul Dutt, both of UF/IFAS, which graft the best GMO and non-GMO scions onto the best GMO rootstocks. This is a particular favorite of mine because I can't shake the feeling that it will take transgenic work to get this problem behind us once and for all.

11. Dr. Amit Levy has a credible and logical hypothesis for getting more OTC into the phloem, where CLAs lives. He adds a natural product that is familiar to the tree to the OTC solution. I can't share the product with you because of Intellectual Property issues, but Dr. Levy may have a simple solution to a daunting problem: how to get a killing agent through the wall of the phloem to kill CLAs.

12. Drs. Kirsten Pelz-Stelinski and Lucasz Stelinski are testing the effects of OTC injection on psyllid pathogenicity and vector populations. While a longshot, wouldn't it be something if OTC made "hot" psyllids less hot, reducing the transference of the disease?

These are just 12 of the exciting projects funded by CRDF. Scientific advancements we haven't even imagined are right around the corner, so CRDF will continue looking for and funding the latest ideas as we push bravely into the new year.

Rick Dantzler, COO, CRDF



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